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ORIGINAL ARTICLE

Variations of CHROMagar Acinetobacter to detect imipenem-resistant Acinetobacter baumannii–calcoaceticus complex

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Abstract

Background: Acinetobacter baumannii–calcoaceticus complex (ABC) isolates are often multidrug-resistant, including to carbapenems. Chromogenic media can facilitate the rapid detection of Gram-negative bacteria, often with the addition of supplements to a base chromogenic medium to detect resistance. We examined various combinations of available media to detect imipenem resistance among 107 ABC clinical isolates. *Methods:* CHROMagar Orientation, CHROMagar KPC, and CHROMagar Acinetobacter, by itself, with Acinetobacter supplement, with KPC supplement, or CHROMagar Acinetobacter with increasing concentrations (1, 2.5, and 5 ml/l) of a new CR102 supplement, were examined. *Results:* Sensitivity for the detection of isolates was high ($>98\%$) for all formulations. Specificity was high for CHROMagar Acinetobacter with 2.5 ml/l and 5 ml/l of the CR102 supplement, at 95.3% and 97.7%, respectively, with positive predictive values of 97% and 98.5%. Negative predictive values of these 2 formulations were 100%. *Conclusions:* CHROMagar Acinetobacter with the addition of the CR102 supplement at 2.5 ml/l and 5ml/l is highly sensitive and specific for the detection of imipenem-resistant ABC, and may be useful for the rapid detection of imipenem-resistant ABC in clinical samples.

Keywords: *Acinetobacter baumannii–calcoaceticus complex, MDR, CHROMagar, carbapenem*

Introduction

Acinetobacter baumannii–calcoaceticus complex (ABC) is a prevalent multidrug-resistant (MDR) nosocomial pathogen with increasing carbapenem resistance, presenting significant treatment and infection control challenges [1–5]. Nosocomial outbreaks of ABC can lead to prolonged hospitalization and possibly increased mortality rates [4]. ABC was the leading MDR organism recovered over a 6-y period in a military burn center [6], and is an important pathogen with multiple resistance mechanisms to antimicrobial agents [2,3,7–9]. As a means of interrupting nosocomial transmission, rapid detection methods providing early identification of MDR ABC would be helpful to provide appropriate, timely treatment and implement adequate infection control measures [3,10].

Chromogenic selective media have been used for the early detection of multiple organisms. These media aid in the rapid detection of pathogens, providing the opportunity for earlier treatment and to guide infection control measures. Chromogenic media are commercially available for the detection of methicillin-resistant Staphylococcus aureus (MRSA) [11–13], vancomycin-resistant Enterococcus spp. [14], extended-spectrum beta-lactamase (ESBL)-producing Gram-negative pathogens [15,16], and

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Candida species [17]. In its initial formulations, CHROMagar Acinetobacter (CHROMagar, Paris, France) showed some promise in the identification of a single clone in the setting of an outbreak [18], but was found to be non-specific for the detection of imipenem-resistant ABC [19,20]. Recently, the addition of a proprietary supplement from a separate medium, CHROMagar KPC, intended to suppress the growth of carbapenem-susceptible bacteria, was shown to improve the specificity of this CHROMagar Acinetobacter using a limited number of ABC study isolates [21]. CHROMagar KPC reliably distinguishes carbapenem-resistant Enterobacteriaceae carrying the KPC gene [22], but was subsequently shown to be less sensitive for the detection of isolates having a lower level of carbapenem resistance [23].

We first examined whether we could replicate the improved diagnostic utility of CHROMagar Acinetobacter base plus Acinetobacter supplement with the KPC supplement for detection of imipenem-resistant isolates compared to non-supplemented CHROMagar Orientation base medium, CHROMagar Acinetobacter base and CHROMagar KPC using a larger number of well-characterized ABC clinical isolates. We then examined CHROMagar Acinetobacter base supplemented with CR102, a proprietary agent designed to improve the selective recovery of carbapenem-resistant organisms, and included in the final medium composition [24], for its ability to detect imipenem-resistant ABC.

Materials and methods

Clinical Acinetobacter isolates

One hundred and seven single-patient ABC isolates from blood and wound infections from 2006 to 2008 were studied. All isolates had previously been characterized by pulsed-field gel electrophoresis (PFGE) typing and had known carbapenem susceptibilities [25,26]. We chose imipenem as the representative carbapenem since it had the greatest activity against these isolates according to Clinical and Laboratory Standards Institute (CLSI) broth microdilution testing. CLSI minimum inhibitory concentration (MIC) breakpoints for imipenem against A. baumannii were used: susceptible ≤ 4 μg/ml, intermediate 8 μg/ml, and resistant ≥ 16 μg/ml [27]. To extend the applicability of study results to Europe, interpretive criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for imipenem were also applied: susceptible ≤ 2 μg/ml and resistant > 8 μg/ml [28].

Control isolates

Twenty-four clinical and reference isolates of Gram-negative and Gram-positive bacteria, including ATCC strains of A. baumannii (ATCC 19606), Acinetobacter lwoffii (ATCC 15309), and A. calcoaceticus (ATCC 23055, ATCC 51432) were tested. Gram-negative control isolates were characterized using Phoenix NMIC/ID 121 or 123 panels (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and VITEK-2/GN25 cards (bioMérieux, NC, USA) to determine imipenem susceptibilities. Susceptibilities of all Gram-positive bacterial isolates were determined by Etest (AB bioMérieux, Solna, Sweden).

Preparation of media

CHROMagar Orientation (CA/O) base medium and CHROMagar Acinetobacter (CA/ABC) base medium were prepared from dehydrated powder in accordance with the manufacturer's instructions. CHROMagar KPC (CA/O + KPC) was prepared as intended by adding the KPC supplement to the dehydrated CA/O base medium. CHROMagar Acinetobacter plus Acinetobacter supplement (CA/ABC + ABC) and CHROMagar Acinetobacter plus KPC supplement (CA/ABC + ABC + KPC) were also prepared in the same manner. In the second phase of the experiment, CA/ABC was supplemented with the CR102 supplement in increasing concentrations (1 ml/l (CA/ABC + CR102-1), 2.5 ml/l

Table I. Description of tested media.

Tested media	Chromogenic base media	Supplement	Additional supplement
CA/O	CHROMagar Orientation	N/A	N/A
CA/ABC	CHROMagar Acinetobacter	N/A	N/A
CA/ABC + ABC	CHROMagar Acinetobacter	Acinetobacter	N/A
CA/ABC + ABC + KPC	CHROMagar Acinetobacter	Acinetobacter	KPC
CA/O + KPC	CHROMagar Orientation	KPC	N/A
CA/ABC + CR102-1	CHROMagar Acinetobacter	1 ml/l CR102	N/A
CA/ABC + CR102-2	CHROMagar Acinetobacter	2.5 ml/l CR102	N/A
CA/ABC + CR102-3	CHROMagar Acinetobacter	5 ml/l CR102	N/A

N/A, Not applicable.

(CA/ABC + CR102-2), and 5 ml/l (CA/ABC + CR102-3)), in accordance with the manufacturer's instructions (Table I). Media were refrigerated, protected from the light, and used within 35 days, as recommended.

Inoculation of media

Bacterial isolates stored at $-80°C$ were passed twice on tryptic soy agar (TSA) containing 5% sheep's blood (REMEL, Lenexa, KS) and incubated at 35°C for 24 h. Saline bacterial suspensions containing approximately 10^8 CFU/ml were prepared using a 0.5 McFarland standard. A 50-μl aliquot of appropriate dilutions, resulting in approximately 50 CFU of each sample, was inoculated onto chromogenic agar plates and incubated at 35°C for 24 h. The color of growing colonies was matched to an artist's color wheel (The Color Wheel Company, Philomath, OR, USA) by 2 independent observers. A single run of CA/O, CA/O + KPC, CA/ABC, CA/ABC + ABC, and CA/ABC + ABC + KPC, was performed to assess the ABC and KPC supplements. For the second phase, a single run of CA/ABC as the control, CA/ABC + CR102-1, CA/ABC + CR102-2, and CA/ABC + CR102-3 was performed.

Polymicrobial cultures

The agar formulations were also tested to assess their ability to distinguish Acinetobacter from polymicrobial inoculae. Thirteen plates of each medium were prepared. Twenty control isolates including clinical and reference strains, and 4 different, randomly selected clinical ABC isolates were selected in groups of 3 isolates and suspended in saline in equal proportions. A 50-μl aliquot of sample, yielding approximately 50 CFU of mixed species, was applied to the agar plates by a person not responsible for data collection or interpretation. These were also plated on TSA for colony counts and confirmation of species present. After overnight incubation at 35°C, the plates were scored for ABC growth and color independently by 2 observers blinded to the composition of the inoculae and the agar formulations.

Statistical methods

For ABC isolates, sensitivity and specificity of the media for the detection of imipenem-resistant isolates (growth of isolates at intended red color) were calculated, as well as positive predictive values (PPV) and negative predictive values (NPV).

Table II. Growth of clinical Acinetobacter baumannii–calcoaceticus complex isolates on tested media.

	Number (%) of isolates that grew with the appropriate appearance on:							
Clinical isolates (number)	CA/O	CA/O + KPC	CA/ABC[a]	CA/ABC + ABC	CA/ABC + ABC + KPC	CA/ABC + CR102-1	CA/ABC + CR102-2	CA/ABC + CR102-3
Acinetobacter baumannii–calcoaceticus complex (107)	107 (100%)	99 (93%)	107 (100%)	106 (99%)	97 (91%)	87 (81%)	66 (62%)	65 (61%)
Imipenem-susceptible (40) (18 PFTs)	40 (100%)	33 (80%)	40 (100%)	39 (98%)	32 (78%)	20 (50%)	0 (0%)	0 (0%)
Imipenem-intermediate (3) (3 PFTs)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	2 (66%)	1 (33%)
Imipenem-resistant (64) (9 PFTs)	64 (100%)	64 (100%)	64 (100%)	64 (100%)	63 (98%)	64 (100%)	64 (100%)	64 (100%)

PFT, pulsed-field type.
[a]CA/ABC was run during each phase of the experiment and results were identical during each run.

Table III. Sensitivity, specificity, positive predictive value, and negative predictive value of CHROMagar formulations for the identification of imipenem-resistant Acinetobacter baumannii–calcoaceticus complex.

	CA/O	CA/O + KPC	CA/ABC	CA/ABC + ABC	CA/ABC + ABC + KPC	CA/ABC + CR102-1	CA/ABC + CR102-2	CA/ABC + CR102-3
Sensitivity	100%	100%	100%	100%	98%	100%	100%	100%
Specificity	0%	0%	0%	2.3%	20%	46.5%	95.3%	97.7%
PPV	60.4%	64.6%	60.4%	60.4%	64%	73.6%	97.0%	98.5%
NPV	100%	100%	100%	100%	88.9%	100%	100%	100%

PPV, positive predictive value; NPV, negative predictive value.

Results

Clinical Acinetobacter isolates

One hundred and seven (100%) of the Acinetobacter clinical isolates produced red colonies on CA-ABC. Using CLSI interpretive criteria, 64 were resistant (IMP-R), 40 were susceptible, and 3 were intermediate to imipenem. Ninety-three percent of all isolates grew on CA/O + KPC, and 91% grew on CA/ABC + ABC + KPC (including susceptible isolates). None of the imipenem-susceptible isolates grew on CA/ABC + CR102-2 and CA/ABC + CR102-3 (Table II). Applying CLSI criteria, sensitivity for the detection of IMP-R isolates was $>98\%$ for all medium formulations, while specificity was highest with increasing CR102 concentration (Table III, Figure 1). CA/ABC + CR102-3 also had the highest PPV, while NPV was high for all the media. Applying EUCAST susceptibility criteria, sensitivities and specificities of the media in the second run were: CA/ABC 100%/0%, CA/ABC + CR102-1 100%/52.5%, CA/ABC + CR102-2 97%/100%, and CA/ABC + CR102-3 95.5%/100%.

Control isolates

Out of 24 control isolates, only 1 reference isolate, A. calcoaceticus ATCC 51432, demonstrated growth of red colonies on the majority of the media tested, but was suppressed on CA/ABC + CR102-2 and CA/ABC + CR102-3. The other reference Acinetobacter isolates, with retained susceptibility to imipenem, did not grow on any media (Table IV). Growth of the other control organisms was infrequently noted, and no other organisms grew on media supplemented with CR102.

Polymicrobial cultures

In the first blinded reading trial, identification of ABC in mixed cultures had a sensitivity/specificity of 100%/60% on CA/O, 80%/67% on CA/O + KPC, 90%/67% on CA/ABC, 90%/67% on CA/ABC + ABC, and 80%/100% on CA/ABC + ABC + KPC. One imipenem-susceptible clinical ABC isolate randomly selected for this portion of the study did not grow on these media in pure culture. In the second

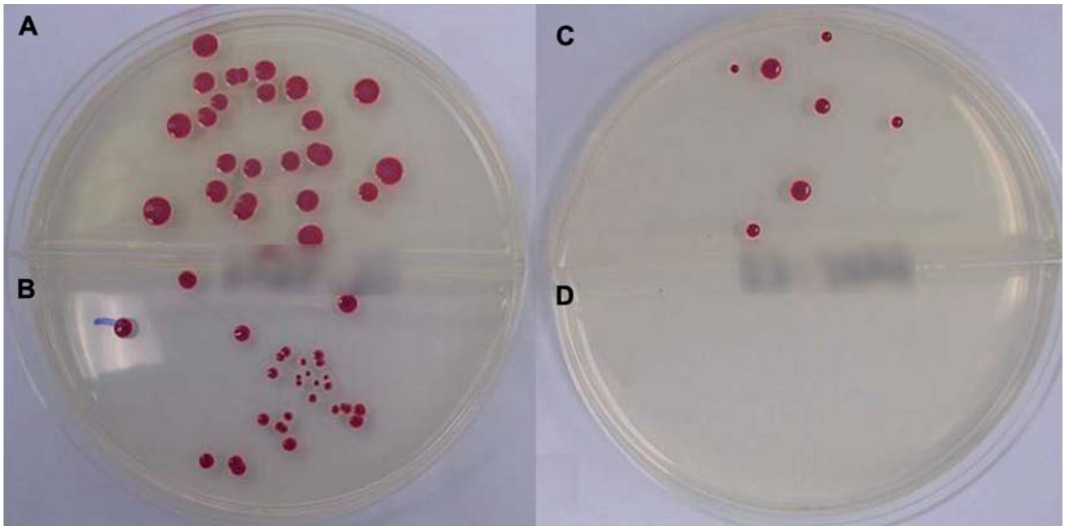

Figure 1. Colonies of an imipenem-intermediate (MIC 8 μg/ml) clinical isolate of Acinetobacter baumannii–calcoaceticus complex showing robust growth on CA/ABC (A: CHROMagar Acinetobacter), decreasing growth with increasing concentrations of CR102 supplement (B: CA/ABC + CR102-1, 1 ml/l CR102; C: CA/ABC + CR102-2, 2.5 ml/l CR102; D: CA/ABC + CR102-3, 5 ml/l CR102).

Table IV. Colony color of the control isolates used for testing the experimental media.

Isolate	Imipenem susceptibility (by CLSI)	CA/O	CA/O + KPC	CA/ABC	CA/AB + ABC	CA/ABC + ABC + KPC	CA/ABC + CR102-1	CA/ABC + CR102-2	CA/ABC + CR102-3
Acinetobacter baumannii ATCC 19606	S	White	NG	NG	NG	NG	NG	NG	NG
Acinetobacter calcoaceticus ATCC 23055	S	NG	NG	NG	NG	NG	NG	NG	NG
A. calcoaceticus ATCC 51432	R	White	Translucent	Red	Red	Red	Red	NG	NG
Acinetobacter lwoffii ATCC 15309	S	White	NG	NG	NG	NG	NG	NG	NG
Citrobacter freundii 971	S	Blue–violet	Red–violet	NG	NG	NG	NG	NG	NG
Enterobacter cloacae, clinical isolate 1	I	Red–violet	Red–violet	NG	NG	NG	NG	NG	NG
E. cloacae 973	S	Blue	NG	Blue	Blue	NG	NG	NG	NG
E. cloacae, clinical isolate 2	S	Blue	NG	White, red[b]	Blue–violet	NG	NG	NG	NG
Escherichia coli, clinical isolate 1	R	Green, blue–green[c]	Red–violet	NG	NG	NG	NG	NG	NG
E. coli, clinical isolate 2	S	Red–violet	NG	NG	NG	NG	NG	NG	NG
E. coli ATCC 25922	S	Red–violet	NG	NG	NG	NG	NG	NG	NG
E. coli ATCC 35218	S	Red–violet	NG	NG	NG	NG	NG	NG	NG
Enterococcus faecalis ATCC 29212	2 μg/ml[a]	Blue–green	NG	NG	NG	NG	NG	NG	NG
E. faecalis ATCC 51299	3 μg/ml[a]	Green	NG	NG	NG	NG	NG	NG	NG
Klebsiella pneumoniae 3142	S	Blue	NG	NG	NG	NG	NG	NG	NG
K. pneumoniae KPC-2	I	Blue	Blue–green	Blue, white[b]	Blue–green	Blue	NG	NG	NG
K. pneumoniae, clinical isolate	S	Blue	NG	Blue	Blue	NG	NG	NG	NG
Pseudomonas aeruginosa, clinical isolate 1	S	White, blue[b]	NG	NG	NG	NG	NG	NG	NG
P. aeruginosa, clinical isolate 2	R	White	Translucent	NG	NG	NG	NG	NG	NG
P. aeruginosa ATCC 27853	S	White	NG	NG	NG	NG	NG	NG	NG
Staphylococcus aureus, clinical isolate (MRSA)	N/A	White	NG	NG	NG	NG	NG	NG	NG
S. aureus ATCC 25923	S	White	NG	NG	NG	NG	NG	NG	NG
S. aureus ATCC 29213	S	White	NG	NG	NG	NG	NG	NG	NG
Serratia marcescens 972	S	Blue–green, white[b]	Translucent	NG	NG	NG	NG	NG	NG

CLSI, Clinical and Laboratory Standards Institute; MRSA, methicillin-resistant Staphylococcus aureus; N/A, not available; NG, no growth.
[a]Minimum inhibitory concentration (MIC).
[b]Denotes more than 1 color of colony growth.
[c]Denotes inter-observer variability.

blinded reading trial, all agars tested (CA/ABC, CA/ABC + CR102-1, CA/ABC + CR102-2, CA/ABC + CR102-3), had sensitivities and specificities of 100% for all tested media. Examples of polymicrobial plates are shown in Figure 2.

Discussion

The development of a reliable medium to more rapidly detect MDR ABC would be helpful to provide a timely and appropriate clinical and infection control response. The goal of this study was to determine the diagnostic characteristics of various CHROMagar formulations using a library of genetically heterogeneous isolates with known carbapenem resistance phenotypes. In this study, we demonstrated that CHROMagar Acinetobacter without additional supplement was not specific for imipenem-resistant isolates, as previously described [19,20]. We also found CHROMagar KPC to have insufficient specificity for the detection of imipenem-resistant ABC. In a recent study of 7 Acinetobacter isolates (5 resistant and 2 susceptible to carbapenems), CHROMagar Acinetobacter with KPC supplement successfully differentiated carbapenem-resistant strains on 2 CHROMagar Acinetobacter medium formulations [21]. However, we were unable to confirm these findings using a larger selection of clinical isolates, including 40 imipenem-susceptible isolates. It is unclear by what mechanism the KPC supplement is able to improve the selection of the CHROMagar media for other uses. Of the 36 imipenem-susceptible and imipenem-intermediate Acinetobacter isolates that grew on CA/O + KPC and CA/ABC + ABC + KPC, 34 were susceptible or intermediate to other carbapenems (doripenem and/or meropenem) and 6 were susceptible or intermediate to ceftazidime. This would suggest that the KPC supplement does not detect carbapenem resistance by the addition of one of these agents. Based on our findings, CHROMagar Acinetobacter with the addition of KPC supplement would have limited utility in detecting carbapenem-resistant ABC.

In contrast, we found that CHROMagar Acinetobacter containing 2.5 ml/l or 5 ml/l (the intended concentration as per the manufacturer) of the proprietary supplement CR102 had high sensitivity and specificity for the detection of imipenem-resistant isolates of ABC, as defined by using both CLSI and EUCAST MIC breakpoints for imipenem against A. baumannii. These performance characteristics rival those of other chromogenic media currently available for clinical use, such as CHROMagar KPC for the detection of carbapenem-resistant Klebsiella pneumoniae (sensitivity/specificity of 100%/98.4%) [22], and CHROMagar MRSA used for the detection of MRSA (sensitivity/specificity $>90\%$) [12]. Given its high sensitivity and specificity for the chromogenic detection of imipenem-resistant ABC in this laboratory-based study, CHROMagar Acinetobacter with CR102 supplementation appears promising for clinical use. While these results are encouraging, and represent a significant improvement over earlier formulations of CHROMagar Acinetobacter, a multicenter clinical trial examining the real-time use of this medium with patient samples submitted to a clinical microbiology laboratory is required before it can be recommended for clinical use.

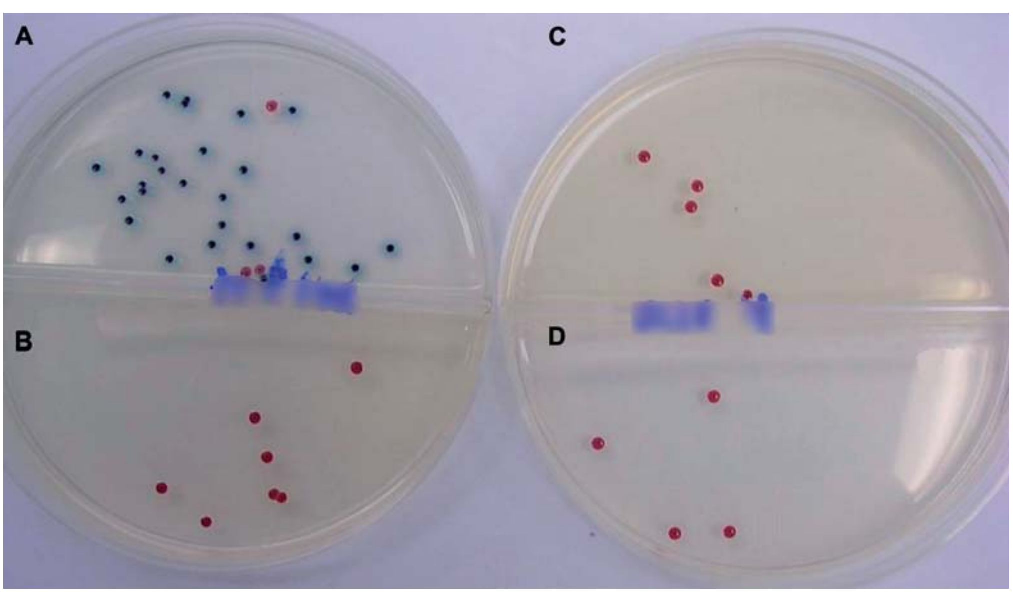

Figure 2. Growth of imipenem-resistant Acinetobacter baumannii–calcoaceticus complex (red colonies, MIC 32 μg/ml) and imipenem-susceptible Enterobacter cloacae (blue colonies, MIC ≤ 1 μg/ml) resulting from a polymicrobial inoculum. B, C and D have evidence of red colony growth consistent with imipenem-resistant Acinetobacter baumannii–calcoaceticus complex. A: CA/ABC, no supplement; B: CA/ABC + CR102-1, 1 ml/l CR102; C: CA/ABC + CR102-2, 2.5 ml/l CR102; D: CA/ABC + CR102-3, 5 ml/l CR102.

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Declaration of interest: Tested media and supplements were obtained from CHROMagar (Paris, France). CHROMagar had no input in study design or data analysis.

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